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DRAPER et al. Appl. No. 09/719,002 (National Phase of International Appl. No. PCT/GB99/01949, filed June 21, 1999)



CROSS REFERENCE TO RELATED APPLICATIONS

The present application is the National Phase of International Application No. PCT/GB99/01949, filed June 21, 1999, which was published in English.

Please replace the first paragraph on page 2 (Table 1) with the following paragraph:

Table 1

Promoter Response Elements Name Sequence Sensitivity **ABRE CCACGTT ABA** Drought DRE1 TACCGACAT E-8 **ATAAGGGGTTGGT** (SEQ ID NO:5) G Box **GTGTCAC** H Box **GGTAGG** JA? (SEQ ID NO:6) JA Box **CCCTATAGGG TGGTTA** Myb Myc **CANNTG** Ethylene PR Box **AGCCGCC TCA TTATCTCCTT** (SEQ ID NO:7)

Please replace the second paragraph on page 5 with the following paragraph:



A number of elements present in PR gene promoters have been identified. The PR-2d gene (encoding a β-1,3-glucanase) from tobacco is expressed in tissue undergoing hypersensitive response (HR) following tobacco mosaic virus (TMV) challenge and is induced by exogenous SA (Shah et al., Plant J. 10:1089-1101 (1996)). Region -364 to -288 in the PR-2d promoter

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confers SA sensitivity and a 25 bp element in this region is recognised by nuclear factors from tobacco. An SA responsive element has also been isolated from the CaMV 35S promoter at position -90 to -46. The element corresponds to an as-1 site (Qin *et al.*, *Plant Cell 6*:863-874 (1994)). The sequence TCATCTTCTT (SEQ ID NO:8) is repeated several times in the barley β-1,3-glucanase promoter and is present in over 30 stress-induced genes (Goldsbrough *et al.*, *Plant J.* 3(4):563-571 (1993b)). This region binds 40 kDa tobacco nuclear proteins, the binding of which is increased in SA-treated plants. Buttner *et al.*, *Proc. Natl. Acad. Sci. USA 94*:5961-5966 (1997) have shown that Arabidopsis ethylene responsive element binding proteins bind to the PR box and that PR- and G-boxes exhibit synergistic effects.

Please replace the third paragraph on page 28 with the following paragraph:



FIGURE 1 shows the Nucleotide sequence of AoPRT-L cDNA (SEQ ID NO:2) together with the predicted amino-acid sequence of AoPRT-L (SEQ ID NO:3). The sequences and positions of binding of the primers used for IPCR (SEQ ID NO:4 and SEQ ID NO:10, respectively) are indicated above the cDNA sequence and relevant enzyme restriction sites underlined.

Please replace the last paragraph on page 28 with the following paragraph:



FIGURE 6 shows the Nucleotide sequence of the AoPRT-L promoter (SEQ ID NO:1). Sequences with homology to characterised promoter elements are boxed.

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Please replace the last paragraph on page 31 with the following paragraph:



P1 5'-CGCGGAATTCGGTGTAGGTGCATTTGTTGG-3' (SEQ ID NO:9) (105-86 bp) and EcoRI

Please replace the first paragraph on page 32 with the following paragraph:



P2 5'-CGCCTGCAGCCAATCCTGGACCCTCACCG-3' (SEQ ID NO:10) (152-172 bp)

PstI

Please replace the last two paragraphs on page 32 with the following paragraphs:



5'- OGGTACCAAGCTTCTTATTGCGACCTGACTCTC 3' (SEQ ID NO:11)

KpnI HindIII

5'- CGCGGATCCGTCGACCTGCAGGATTGGTTGTTGTTTTT 3' (SEQ ID NO:12)

BamHI SalI PstI

Please replace the second full paragraph on page 40 (Example 12) with the following paragraph:



Example 12 - Identification and multimerisation of an SA/BTH responsive element in the

AoPRT-L promoter.

A series of 3 AoPRT-L 5' promoter deletion - GUS fusion constructs were constructed using the following primers designed to regions of the AoPRT-L promoter (Figure 15a):-

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- 5' GCG<u>AAGCTT</u>CCATGTCATGAGAGAAGCAC 3' (-361 bp) (SEQ ID NO:13) HindIII
- 5' GCGAAGCTTTTGGAAACTGAATACCTACA 3' (-247 bp) (SEQ ID NO:14) HindIII
- 5' GCGAAGCTTACAAAGGCTTAGACTTTCCA 3' (-132bp) (SEQ ID NO:15) HindIII

Each of the above primers, in conjunction with the primer below, was used in a PCR reaction with p22-JIT60 as template:-

5' GGGATCCGTCGACCTGCAGATTGGTTGTTGTTTTTTG 3' (SEQ ID NO:16)

BamHI

SalI

PstI

Please replace the second paragraph on page 41 with the following paragraph:

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In order to construct an AoPRT-L promoter that has higher expression, the region -247 bp to -

133 bp was amplified from p22-JIT60 and placed twice in front of a -247 bp

AoPRT-L promoter. This AoPRT-Lx3 promoter was constructed as follows:- The primers below were used to PCR the 0bp to -247bp AoPRT-L promoter from p22-JIT60.

5'-TCTAGGTACCCTTTGCGTGGTCGACTTGGAAACTGAATACCTAC-3' (SEQ ID NO:17)

KpnI SalI

 $5°G\underline{GGATCCGTCGACCTGCAG}ATTGGTTGTTGTTTTTTG\ 3°(SEQ\ ID\ NO: 16)$

BamHI SalI PstI

This was cloned as a KpnI, PstI fragment into pUC19. The 133bp to-247bp pAoPRT-L region was amplified with the primers:-

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 $5°TCTA\underline{GGTACC}CTTTGCGTG\underline{GTCGAC}TTGGAAACTGAATACCTAC~3°(SEQ~ID~NO:18)$

KpnI

SalI

5'GAAAGTCTAAGC<u>CTCGAG</u>GGAATAAGGTACGAGTTCGTGGAC 3'(SEQ ID NO:19) XhoI